

## THE EFFECTS OF TEMPERATURE AND SALINITY ON LARVAL GROWTH OF THE HORSESHOE CRAB *LIMULUS POLYPHEMUS*

ROY LAUGHLIN

*University of California, Naval Biosciences Laboratory, Naval Supply Center, Oakland, CA 94625*

### ABSTRACT

Horseshoe crab, *Limulus polyphemus*, eggs were reared through the first tailed stage in factorial combinations of temperature and salinity with respective values of 20, 25, 30, or 35°C and 10, 15, 25, or 35‰. Lowest survival occurred in low temperature, low salinity combinations (20°C, 10‰). Wet weight, and thus linear dimension, of the first tailed stage was only slightly different throughout the temperature-salinity range tested. However, ash-free dry weights, indicating yolk utilization, varied significantly with both temperature and salinity. The least yolk utilization occurred at 30°C and increased in higher and especially lower temperatures. Yolk utilization also increased with a decrease in salinity. Temperature interacted with salinity to cause a greater salinity-dependent difference in yolk utilization at lower temperatures. Comparisons of ash-free dry weights (*i.e.*, yolk utilization) with respiration rates indicate that *L. polyphemus* has little ability to compensate metabolically for the effects of temperature and, secondarily, of salinity. Nevertheless, the larvae are sufficiently provisioned with yolk to survive the prevailing ranges of these variables in the habitat where adults normally deposit eggs. This suggests that other unexamined physical variables, or more likely biological factors such as predation or competition among the feeding larval stages and adults, are important for larval survival of *L. polyphemus*.

### INTRODUCTION

The brooding behavior of the horseshoe crab, *Limulus polyphemus*, is unique among marine macrofaunal arthropods. A brooding pair in amplexus deposits the eggs in moist sand at or near the water line (Lockwood, 1870; Packard, 1872). Depending primarily upon temperature, the eggs develop for a period of weeks or perhaps months to the trilobite, the first free-living stage (Jegla and Costlow, 1979; 1982). The trilobites generally remain close to the shore, but may also join the plankton (Rudloe, 1979, 1981). The first description of the trilobite stage in the scientific literature was based on material collected from the plankton (see Packard, 1872). In the nearshore environment, both physical and trophic factors are unpredictable. Anecdotal evidence indicates that the animals are very tolerant of sub-optimal conditions (Kingsley, 1892), including low salinity (Mangum *et al.*, 1976). Since the trilobite depends on stored yolk material and does not feed, it is protected from trophic instability until after the molt to the first tailed stage.

The ability to tolerate a range of physical factors exacts some cost in terms of metabolic adjustment which must be integrated within the constraints imposed by the amount of energy and material originally supplied within the egg. If development were excessively slowed by low temperatures or osmotic regulation requirements, then growth and perhaps survival would be influenced adversely by these suboptimal

conditions. The experiments reported here were designed to determine the ranges of temperature and salinity over which development to the first tailed stage would occur. Oxygen consumption rates were measured in order to compare the effects of physical factors on energy production and development rate. Following molting to the first tailed stage, the ash-free dry weights were determined. Differences in ash-free dry weight reflect relative amounts of yolk utilized during development.

#### MATERIALS AND METHODS

The eggs used in this experiment were collected in June, 1979 near Panacea, Florida (USA) by the Gulf Specimen Company and shipped to California by air. Two clutches showing the least development were selected. Based on the complete lack of morphological differentiation in the eggs and the amount of time required subsequently for development, the eggs were probably less than 1 wk old at the initiation of the experiment, and there was probably approximately a 2 day difference in the age of the two clutches.

During shipment, the eggs were in 25‰ sea water. Previous experience had indicated that the developing embryos were quite tolerant of salinity and temperature changes. On the first day of the experiment the eggs were divided into the experimental groups and put directly into the test temperature-salinity combinations with the exception of the 10‰ groups. These were put into 15‰ for the first 24 h. The embryos were exposed to factorial combinations of temperature (20, 25, 30, or 35°C) and salinity (10, 15, 25, or 35‰) for the remainder of larval development to the first tailed stage.

Oxygen consumption rates were determined for free-living trilobites under all temperature-salinity combinations. Individuals for respirometry were not selected until after all the trilobites emerged from their hatching membranes. Oxygen consumption rates were determined using the oxygen electrode in a Lexington Instruments (Lexington, Massachusetts, USA) blood gas analyzer (Laughlin *et al.*, 1979).

The sea water was changed 5 days each week and the animals in the bowls were censused daily. As necessary, cast egg shells and embryonic membranes were removed and algae cleaned from the bowls using paper tissue. No food was added since none of the stages used in this experiment required it. As the larvae molted to the third tailed stage, they were removed from the bowls, blotted dry, weighed to the nearest 0.01 mg, and dried for at least 2 days at 60°C before being weighed to the nearest 0.1 µg. Subsequently, the tissue was ashed in a kiln at 475°C and the ash weight determined to the nearest 0.1 µg. All weights were measured with Cahn 21 electrobalance.

The figures give means and standard deviations. Two means whose standard deviations do not overlap are different at the 5% level. Data were analyzed statistically using the regression subroutine in the Statistical Package for the Social Sciences, SPSS, (Nie *et al.*, 1975). A regression model containing quadratic and linear terms for temperature and salinity and the linear interaction term was generated and plotted.

#### RESULTS

Survival to the first stage varied with physical factor combinations. The percent survival is given by the numbers at the base of each histogram column (Fig. 1). Under optimal conditions, more than 50% of the eggs developed to free-living trilobites. In combinations of low temperature (20°C) and low salinity (10 or 15‰) survival was notably poor. There was an unusual pattern of mortality. In several

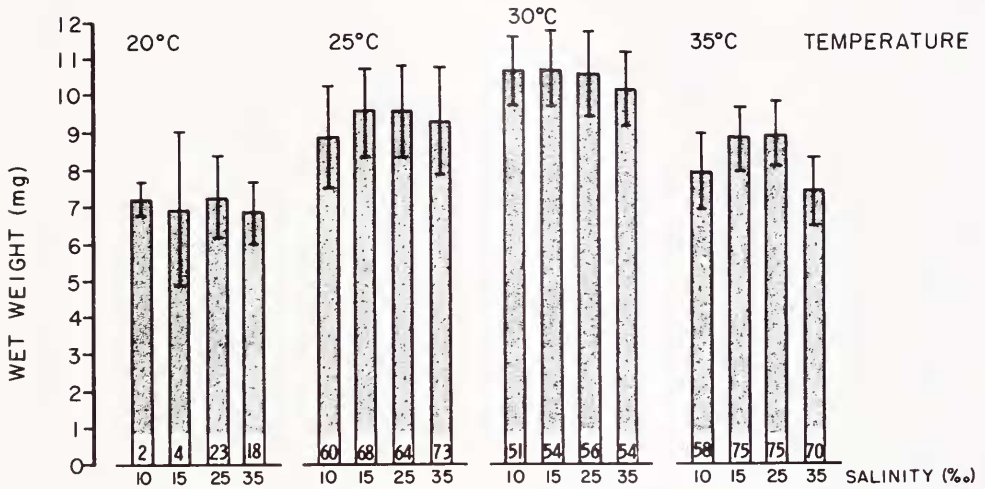


FIGURE 1. Wet weights of first tailed stage *Limulus polyphemus* exposed to factorial combinations of temperature and salinity. The numbers near the base of each column denote the percentage of animals successfully molting to the first tailed stage. Error bars show one standard deviation of the mean. Two hundred eggs were tested in all temperature groups except 35°C where only 40 eggs per salinity were tested.

instances, trilobites survived over 6 wk past the mean time of molting for their group, gradually becoming moribund prior to death. This was especially common at 20°C.

Wet weights varied with both temperature and salinity (Fig. 1). The highest mean wet weights occurred in all salinities at 30°C (~10 mg) and decreased in the order 25° (~9.2), 35°C (~8.5 mg) and 20°C (~7 mg). The effect of salinity was secondary to that of temperature. Within each temperature group, the reductions in wet weight were neither large nor consistent. The largest difference within one temperature (17%) occurred at 35°C, with 25 and 35‰.

Ash-free dry weights, the weights of organic material in the first tailed stage, showed a consistent response to both temperature and salinity at temperatures between 20 and 30°C (Fig. 2). The effect of temperature here was similar to that on wet weights. The largest amount of organic material (~1000 µg per larva) was present in trilobites raised at 30°C. The mean ash free dry weights of larvae at 25 and 35°C were less than at 30°C, with respective values of 900 and 850 µg per larva. The lowest mean weight, 750 µg per larva occurred at 20°C. The effect of salinity on ash-free dry weights was remarkably consistent in all temperatures except 35°C and even here the exceptions were not great. There was a 50–100 µg increase in these weights with a 5–10‰ salinity increase. The relative effect of temperature on the salinity-mediated changes in weight was smallest at 30°C and became progressively larger as the temperature decreased to 20°C. The effect of low salinity on ash-free dry weight is obvious when the differences in mean weights in the 10‰ groups and among the other salinity groups are compared. The 5‰ salinity decrease from 15–10‰ caused a decrease in ash-free dry weight equal to or greater than that caused by a 10‰ change in higher salinities. The growth pattern in 35°C did not show the regular weight increases with salinity which occurred in lower temperatures, and an interaction between temperature and salinity was apparent at 35°C, since the mean ash-free dry weight for the 35‰ salinity group declined approximately 25 µg from that of the 25‰ group.

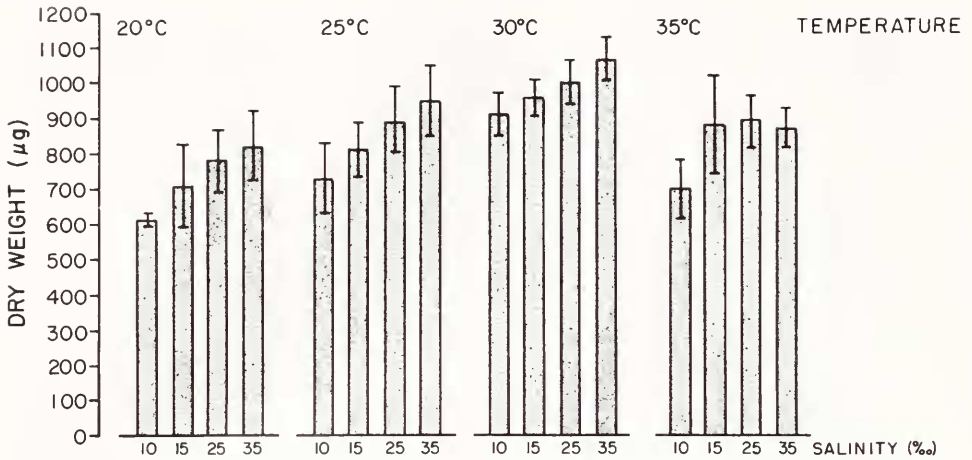


FIGURE 2. Ash-free dry weight of first tailed stage *Limulus polyphemus* exposed to factorial combinations of temperature and salinity. Bars show one standard deviation of the mean.

These data were analyzed by response surface analysis. The following regression equation predicts the effects of temperature and salinity on ash-free dry weight of the trilobites:

$$\text{Ash-free dry weight} = -0.0326 + 0.474T - 0.069T^2 + 0.159S$$

$$- 0.0084S^2 - 0.021T \times S$$

$$R = 0.614$$

$$F = 174$$

$$df = 4, 1155$$

$$p < 0.0001$$

where

T = temperature

S = salinity

R = correlation coefficient

F = F-test ratio

df = degrees of freedom

The response surface shows a ridge of maximum response which is tilted just off and above the 30° isocline (Fig. 3). The ridge descends with decreasing salinity. The effect of low salinity-low temperature combinations is very evident here, with predicted decreases in ash-free dry weights of 45% from a high of 1.00 mg to only 0.55 mg under low temperature-salinity combinations.

Development rate was strongly influenced by temperature (Fig. 4). The shortest mean time required to reach the first tailed stage, 20 days, occurred at 30°C. The 35°C groups required almost a week longer to molt to the first tailed stage. As temperature decreased from 30°C, there was a decrease in development rates and an increase in variability among the groups, indicating an interaction between temperature and salinity. The groups reared in 25°C required between 41 and 51 days to molt, while at 20°C this was dramatically increased to 89–176 days with an even larger range for the different salinity groups. (Some of the trilobites actually survived several more weeks at 20°C before dying without molting).

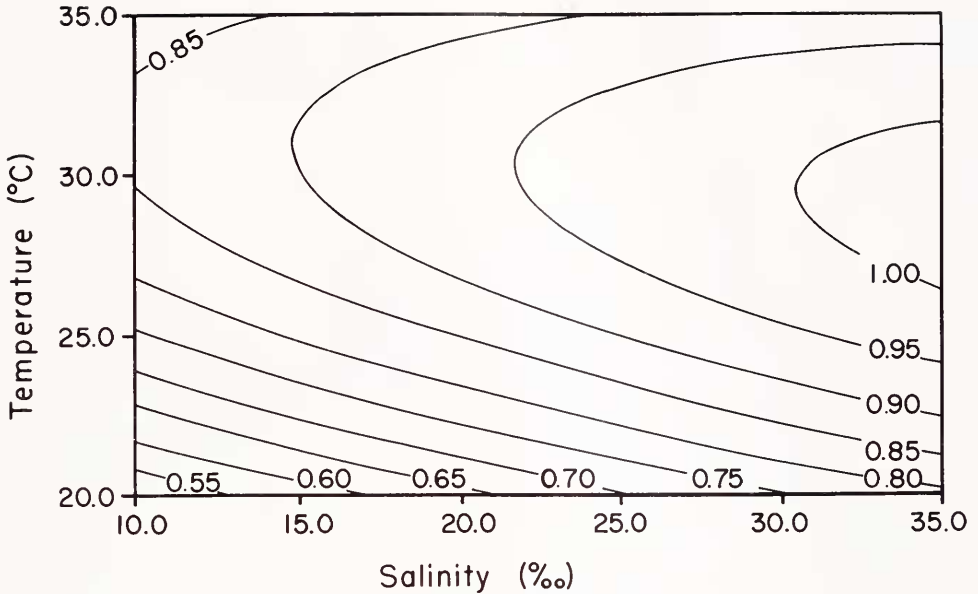


FIGURE 3. Response surface diagram showing predicted ash-free dry weights of first tailed stage *Limulus polyphemus* exposed to factorial combinations of temperature and salinity. Contour units are mg.

Both high (35‰) and low (10‰) salinities had delaying effects on development rate, but the magnitude of the delay was mediated by temperature. At 30°C, the optimum temperature, salinity had little effect on development rates; whereas, at 35°C a slight effect at high and low salinities was apparent. At 25°C development

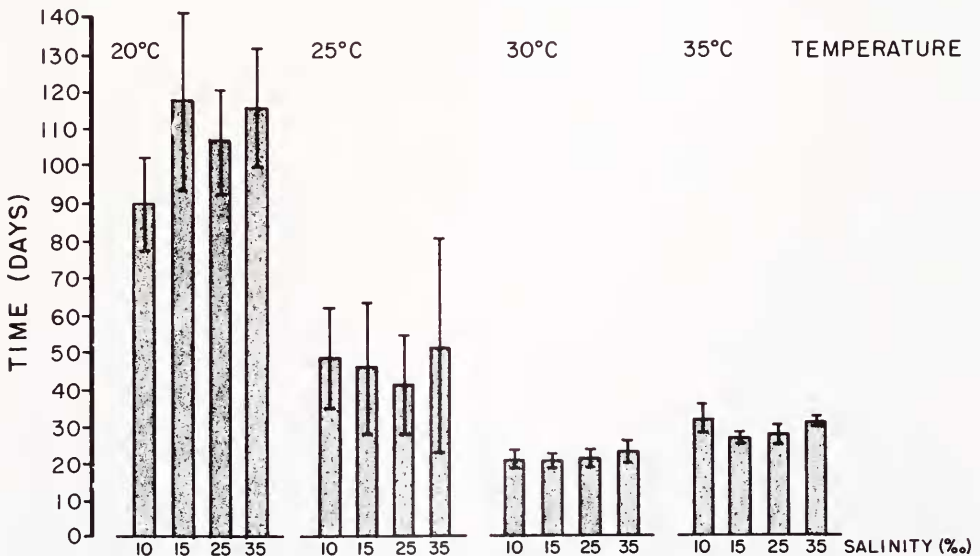


FIGURE 4. Duration of larval development to the first tailed stage of *Limulus polyphemus* exposed to factorial combinations of temperature and salinity. Bars show one standard deviation of the mean.

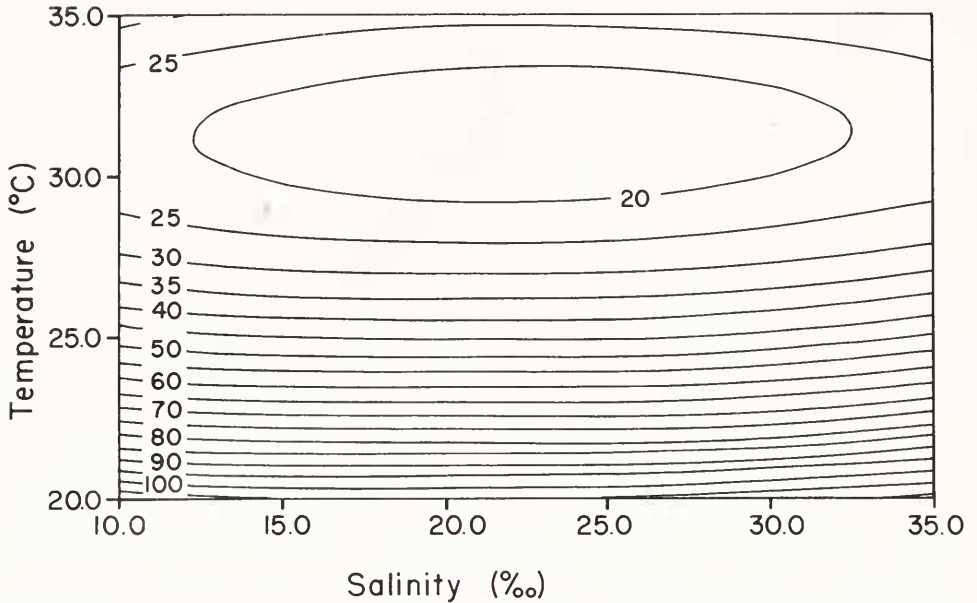


FIGURE 5. Response surface diagram showing effects of temperature and salinity on predicted development rates to the first tailed stage of *Limulus polyphemus*. Contour units are days.

was most rapid in 25‰, decreasing slightly in both higher and lower salinities. The differences were not large, however. At 20°C, temperature was exerting the main effect, expressed as high mortality and very low weights of surviving first tailed stage individuals.

The response surface diagram showing the effects on temperature and salinity (Fig. 5) was plotted from the following regression equation:

$$\text{Days to molt} = 207.2 - 111.6T + 17.5T^2 - 7.2S + 20S^2 - 0.86T \times S$$

$$R = 0.854$$

$$F = 311$$

$$df = 4, 1155$$

$$p < 0.0001$$

where

T = temperature

S = salinity

R = correlation coefficient

F = F-test ratio

df = degrees of freedom

The optimal temperature is predicted to be  $\sim 31^\circ\text{C}$  with a broad salinity optimum. The major axis of the central ellipse is nearly parallel to the salinity axis indicating little interaction between temperature and salinity.

Temperature was the major factor influencing oxygen consumption (Fig. 6). The  $Q_{10}$ 's were generally less than 2 indicating metabolic compensation. This compensation was least effective in the highest (35‰) and lowest (10‰) salinities tested. As

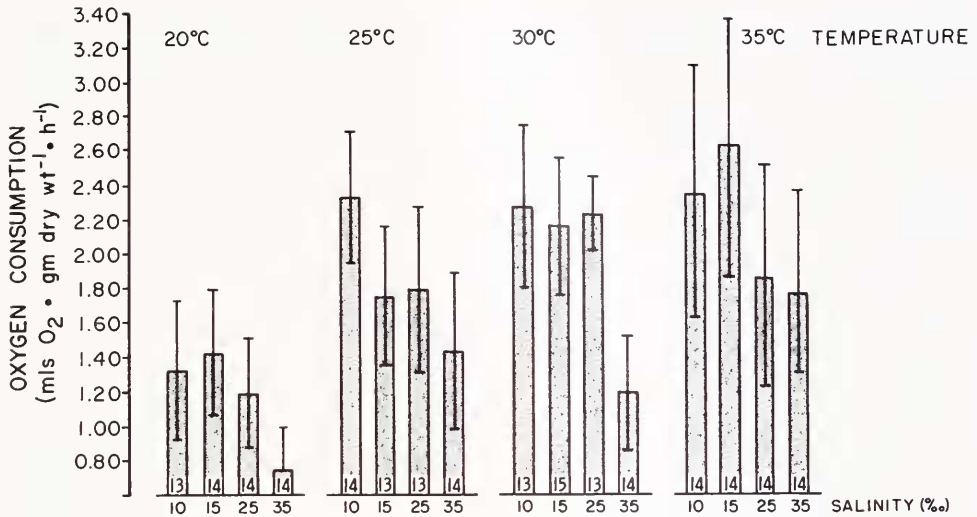


FIGURE 6. Oxygen consumption rates of free-living *Limulus polyphemus* trilobites acclimated to indicated temperature-salinity combinations prior to respirometry. Bars show one standard deviation of the mean. The numbers at the base of each column indicate the number of replicates.

a general trend, the oxygen consumption rates decreased with increasing salinity at each temperature. The relationship of temperature and salinity on oxygen consumption is given by the following equation:

$$\text{Oxygen consumption} = 1.434 + 0.536T - .00083T^2 + .0314S - 0.000745S^2 - 0.0000413T \times S$$

$$R = 0.658$$

$$F = 37.8$$

$$df = 5, 214$$

$$p < 0.001$$

where

T = temperature

S = salinity

R = correlation coefficient

F = F-test ratio

df = degrees of freedom

The temperature, but not the salinity, effect is clearly shown (Fig. 7). The contours are nearly perpendicular to the temperature axis.

## DISCUSSION

Although *Limulus polyphemus* developmental stages tolerate a wide range of temperature-salinity combinations, these data indicate that they are comparatively more sensitive to low temperatures than to low salinities, within the normal environmental ranges. Survival is high, at least in some factor combinations, at tem-

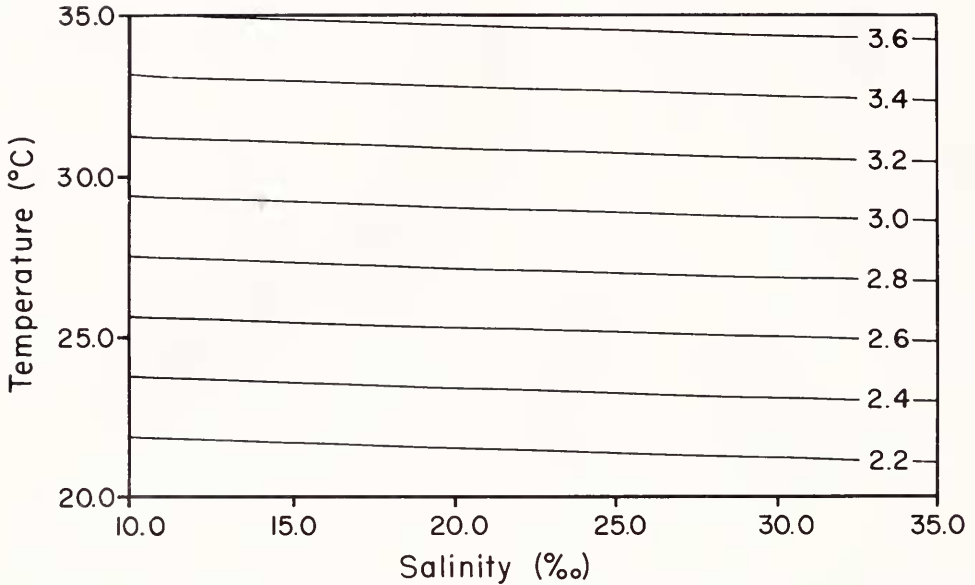


FIGURE 7. Response surface diagram showing predicted oxygen consumption rates of *Limulus polyphemus* acclimated to factorial combinations of temperature and salinity. Contour units are mls O<sub>2</sub> · g dry wt<sup>-1</sup> · h<sup>-1</sup>.

peratures of 20–35°C and salinities of 10–35‰. Sublethal effects on growth and development rate are marked, however.

Wet weights varied with temperature but not nearly to the same extent as with salinity, and overall the differences were not large. The lack of a clear-cut salinity effect is surprising since one might predict a larger animal in lower salinities due to post-molt swelling. However, the yolk loss in low salinities (Fig. 2) produced an initially “smaller” larva which failed to reach larger proportions regardless of any putative differential swelling. Other explanations are also possible since post-molt water uptake mechanisms are not characterized for *Limulus* and may not be similar to those reported for decapods (Mykels, 1980). Ecologically, attainment of a certain minimum dimension may be an important deterrent to predation since many predators (e.g., fish) are highly size-selective for their prey. Rudloe (1981) found correlations between the size of *Limulus polyphemus* juveniles and their distance from the shore. This observation indicates (but does not prove) that younger, smaller horseshoe crabs in shallow water could avoid predators limited by water depth.

Several observations can be cited to show that temperature effects on yolk utilization are mediated primarily by changes in development rate. Oxygen consumption consistently increased over the temperature range tested; however, development rate was most rapid at 30°C, declining slightly at 35°C and markedly as temperature decreased to 20°C. Dry weight of the third tailed stage animals decreased as the duration of development increased. These observations indicate that even at 20°C oxidative metabolism could provide sufficient energy for developmental events. Therefore, there appears to be only a loose coordination between the rate at which energy is supplied and is used to promote development. Hubbs (1926) suggested that a similar lack of coordination between tissue formation and differentiation is the reason that fish embryos reared in colder temperatures generally have more



somites following differentiation events. The situation with *Limulus* represents another paradigm of similar origin. Development rate and oxidative metabolism are best co-ordinated at 30°C, for the population tested, but this decreases in higher or lower temperatures. Rearing at 20°C, the lowest tested here, markedly affected survival, indicating that there is a point of no return. If too much carbon is used for maintenance metabolism before developmental events proceed far enough to allow a molt, the animals cannot molt. This is not a case of simple starvation, however, since the trilobites will live weeks or in some cases months following the mean molting time of successful siblings. The process in *Limulus* may have a similar origin and controlling factor to that studied in starved crab larvae (Anger *et al.*, 1981). While hormonal mechanisms have been suggested, perhaps other mechanisms, at the level of cell metabolism, should be considered since the existence of a point of no return appears to be a salient feature of invertebrate metamorphosis in general (Thorson, 1946, 1966).

The data for *Limulus* show that the effect of temperature on molting, growth, and differentiation is complex. Studies of post-metamorphic fish have provided many experimental models of the effects of temperature on growth, but these are probably inaccurate for *Limulus* or other invertebrate larvae. Alderdice (1972) has reviewed many of the studies on fish and has noted that while growth to a certain size was slower in lower temperatures, growth efficiencies (weight gain per food input) were higher. For *Limulus*, the decelerating effects of low temperature on development rates were more detrimental than any benefit derived from lower maintenance metabolism costs. This is observed for species with feeding as well as non-feeding larvae (Rice and Provenzano, 1965; Provenzano, 1967, 1968; Gore, 1970; Peckenik, 1979; Anger and Dawirs, 1981; Caswell, 1981; Laughlin and Neff, 1981). The occurrence of latitudinal populations of *Limulus*, as well as numerous other species, which develop under very different temperatures shows that temperature sensitivity is under selective control.

There is anecdotal evidence suggesting that juveniles of *Limulus polyphemus* are more salinity tolerant than adults (Mangum *et al.*, 1976), but there is only one study of the mechanisms involved (Laughlin, 1981). The data reported here are for salinities as low as 10‰, but *Limulus* will actually survive and develop to the trilobite stage in salinities as low as 5‰. However, based on the results here, it is doubtful they would survive to the first tailed stage at these low salinities.

Decreasing salinity predictably reduced the ash-free dry weight of the first tailed stage, with the extent of reduction between highest and lowest salinity being mediated secondarily by temperature. Previous studies indicate that loss of carbon is not mediated by costs of ionic regulation. Intracellular osmotic regulation in *Limulus* is mediated by an organic solute which has not been specifically identified (Bricteux-Gregoire *et al.*, 1966). Commitment of organic carbon to osmotic regulation, particularly if it were excreted rather than being "recycled", (for instance by oxidative deamination) in low salinities, could explain the growth pattern with relation to salinity.

The success of *Limulus* larvae, in ecological terms, is dependent upon the choice of breeding beaches where eggs are deposited, as well as the tolerance of larvae to prevailing physical conditions after deposition. I am not aware of any studies specifically addressing the physical factor regime on *Limulus* breeding beaches, but anecdotal evidence and personal experience suggests that adults do not occur where conditions exceed the broad temperature-salinity ranges tolerated by larvae. It is likely that the broad tolerance range of larvae is an adaptation to post-depositional

events which adults do not experience. Rain may significantly dilute salinities, and temperatures may increase with season, particularly along the southeastern coast of North America. Thus, the broad temperature and salinity tolerance range of *Limulus* larvae is not an adaptation allowing exploitation of a large range of habitats within an estuarine system, but rather an insurance against unusually large deviations in temperature and salinity which occur randomly in degree and duration from year to year. The apparently copious stored yolk material endows *Limulus polyphemus* larval stages with a resource base allowing survival in the unpredictably variable estuarine habitat.

#### ACKNOWLEDGMENTS

I thank Henry Bray and Willie French for technical assistance. Sharon Lynn and Caro Hopper assisted with manuscript preparation. This research was supported by the US Navy Office of Naval Research, Contract No. N00014-80-C-0570, NR 205-04.

#### LITERATURE CITED

- ALDERDICE, D. F., 1972. Response of marine poikilotherms to environmental factors acting in concert. Pp. 1659-1722 in *Marine Ecology*, O. Kinne, ed. Vol. 3(1). Wiley Interscience, New York.
- ANGER, K., AND R. R. DAWIRS, 1981. Influence of starvation on the larval development of *Hyas araneus*. *Helgol Meeresunters* **34**: 287-311.
- ANGER, D., R. R. DAWIRS, V. ANGER, J. W. GOY, AND J. D. COSTLOW, 1981. Starvation resistance of first stage zoeae of brachyuran crabs in relation to temperature. *J. Crustacean Biol.* **1**: 518-525.
- BRICTEAUX-GREGOIRE, S., G. DUCHATEAU-BOSSON, C. JUENIAUX, AND M. FLORKIN, 1966. Les constituants osmotiquement actifs des muscles et leur contribution a la regulation isosmotique intracellulaire chez *Limulus polyphemus*. *Comp. Biochem. Physiol.* **19**: 729-736.
- CASWELL, H., 1981. The evolution of "mixed" life histories in marine invertebrates and elsewhere. *Am. Nat.* **117**: 529-536.
- GORE, R. H., 1970. *Petrolisthes armatus*: A redescription of larval development under laboratory conditions (Decapoda, Porcellanidae). *Crustaceana* **18**: 75-89.
- HUBBS, C. L., 1926. The structural consequences of modifications of the development rate in fishes, considered in reference to certain problems of evolution. *Am. Nat.* **60**: 57-81.
- JEGLA, T. C., AND J. D. COSTLOW, 1979. The *Limulus* bioassay for ecdysteroids. *Biol. Bull.*, **156**: 103-114.
- JEGLA, T. C., AND J. D. COSTLOW, 1982. Temperature and salinity effects on developmental and early posthatch stages of *Limulus*. Pp. 103-113 in *Physiology and Biology of Horseshoe Crabs: Studies on Normal and Environmentally Stressed Animals*. Alan R. Liss, New York.
- KINGSLEY, J. S., 1892. The embryology of *Limulus*. *J. Morphol.*, **7**: 35-68.
- LAUGHLIN, R. B., JR., 1981. Sodium, chloride and water exchange in selected developmental stages of the horseshoe crab *Limulus polyphemus* (Linnaeus). *J. Exp. Mar. Biol. Ecol.* **52**: 135-146.
- LAUGHLIN, R. B., JR., AND J. M. NEFF, 1981. Ontogeny of respiratory and growth responses of larval mud crabs *Rhithropanopeus harrisi* exposed to different temperatures, salinities and naphthalene concentrations. *Mar. Ecol. Prog. Ser.* **5**: 319-332.
- LAUGHLIN, R. B., JR., H. W. WOFFORD, AND J. M. NEFF, 1979. A simple potentiometric method for the determination of respiration rates in small aquatic organisms. *Aquaculture*, **16**: 77-82.
- LOCKWOOD, S., 1870. The horsefoot crab. *Am. Nat.* **4**: 257-274.
- MANGUM, C. P., C. E. BOOTH, P. L. DEFUR, N. A. HECKEL, R. P. HENRY, L. C. OGLESBY, AND G. POLITES, 1976. The ionic environment of hemocyanin in *Limulus polyphemus*. *Biol. Bull.* **150**: 453-467.
- MYKELS, D. L., 1980. The mechanism of fluid absorption at ecdysis in the American lobster, *Homarus americanus*. *J. Exp. Biol.* **84**: 89-102.
- NIE, N. H., C. H. HULL, J. G., JENKINS, K., STEINBRENNER, AND D. H. BENT. 1975. *Statistical Package for the Social Sciences*. McGraw-Hill, New York, 675 pp.

- PACKARD, A. S., 1872. The development of *Limulus polyphemus*. *Mem. Boston Soc. Nat. Hist.* **2**: 155-202.
- PECHENIK, J. A., 1979. Role of encapsulation in invertebrate life histories. *Am. Nat.* **114**: 859-870.
- PROVENZANO, A. J., 1967. The zoeal stages and glaucothoe of the tropical eastern Pacific hermit crab *Trizopagurus magnificus* (Bouvier, 1898) (Decapoda, Diogenidae), reared in the laboratory. *Pac. Sci.* **21**: 457-473.
- PROVENZANO, A. J., 1968. The complete larval development of the West Indian hermit crab *Petrochirus diogenes* (L.) (Decapoda, Diogenidae) reared in the laboratory. *Bull. Mar. Sci.* **18**: 143-81.
- RICE, A. L., AND A. J. PROVENZANO, 1965. The zoeal stages and glaucothoe of *Paguristes sericeus* A. Milne-Edwards (Anomura, Diogenidae). *Crustaceana* **8**: 239-254.
- RUDLOE, A., 1979. Locomotor and light responses of larvae of the horseshoe crab, *Limulus polyphemus* (L.). *Biol. Bull.* **157**: 494-505.
- RUDLOE, A., 1981. Aspects of the biology of juvenile horseshoe crabs, *Limulus polyphemus*. *Bull. Mar. Sci.* **31**: 125-133.
- THORSON, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates. *Meddr. Kommn. Habunders (Ser. Plankton)* **4**: 1-523.
- THORSON, G. 1966. Some factors influencing the recruitment and establishment of marine benthic communities. *Neth. J. Sea. Res.* **3**: 267-293.